Supplementary Material

Calpain-1 ablation partially rescues disease-associated hallmarks in models of Machado-Joseph disease

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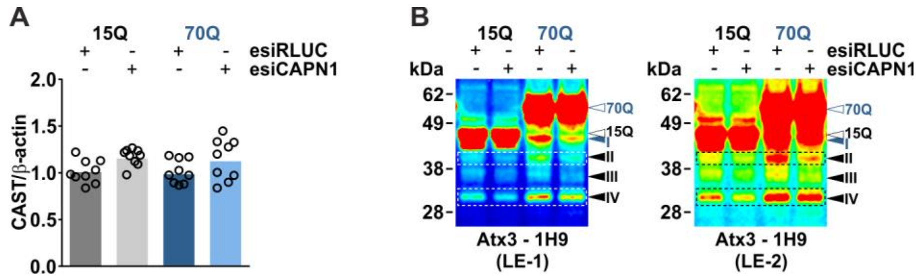
Supplementary Tables

Table S1: Clinical and genetic data of MJD patients and healthy controls

Subject	Gender	*ATXN3* CAG repeat length	Age at symptom onset	Age at biopsy	Internal Code	Purpose
Patient 1	female	70	49 yr	61 yr	AX-25	fibroblasts
Patient 2	male	73	37 yr	41 yr	AX-26	fibroblasts
Patient 3	male	76	21 yr	29 yr	AX-36	fibroblasts
Control 1	female	normal	-	46 yr	CO-53	fibroblasts
Control 2	female	normal	-	37 yr	CO-54	fibroblasts
Control 3	male	normal	-	47 yr	CO-57	fibroblasts

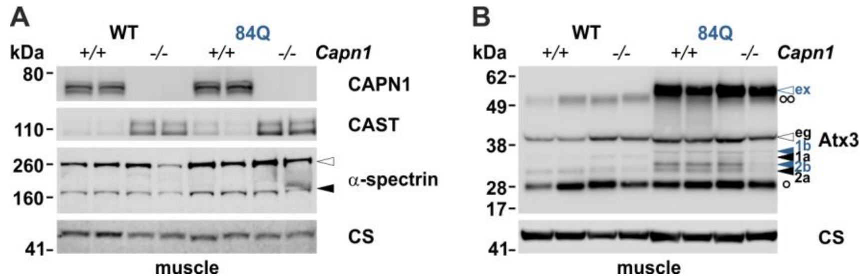
Supplementary Figures and Figure Legends



Supplementary Figure S1. Quantification of CAST levels and detection of ataxin-3 cleavage in MJD cells after knockdown of calpain-1

(A) Densitometric quantification of CAST levels (n = 9) in HEK 293T *ATXN3*$^{-/-}$ cells double-transfected with V5-tagged ataxin-3 15Q or 70Q and esiRNA directed against human calpain-1 (esiCAPN1) or control esiRNA against *Renilla* luciferase (esiRLUC). Columns represent means.

(B) Western blot of ataxin-3 cleavage in *ATXN3*$^{-/-}$ cells double-transfected with V5-tagged ataxin-3 15Q or 70Q and esiRLUC or esiCAPN1. Ataxin-3 breakdown products (bdp) were detected using an antibody specific against the centre (Atx3 - 1H9) of the protein. Two long exposures (LE-1 and LE-2) are shown in false-colours to highlight intensity differences between bands. Fragments analysed via densitometry as shown in Fig. 1H are indicated by white or black dashed line boxes. Full-length ataxin-3 15Q or 70Q is indicated by black or blue-lined white arrowheads, respectively. Black arrowheads indicate N-terminal fragments, the blue one a polyQ-containing ataxin-3 breakdown product.

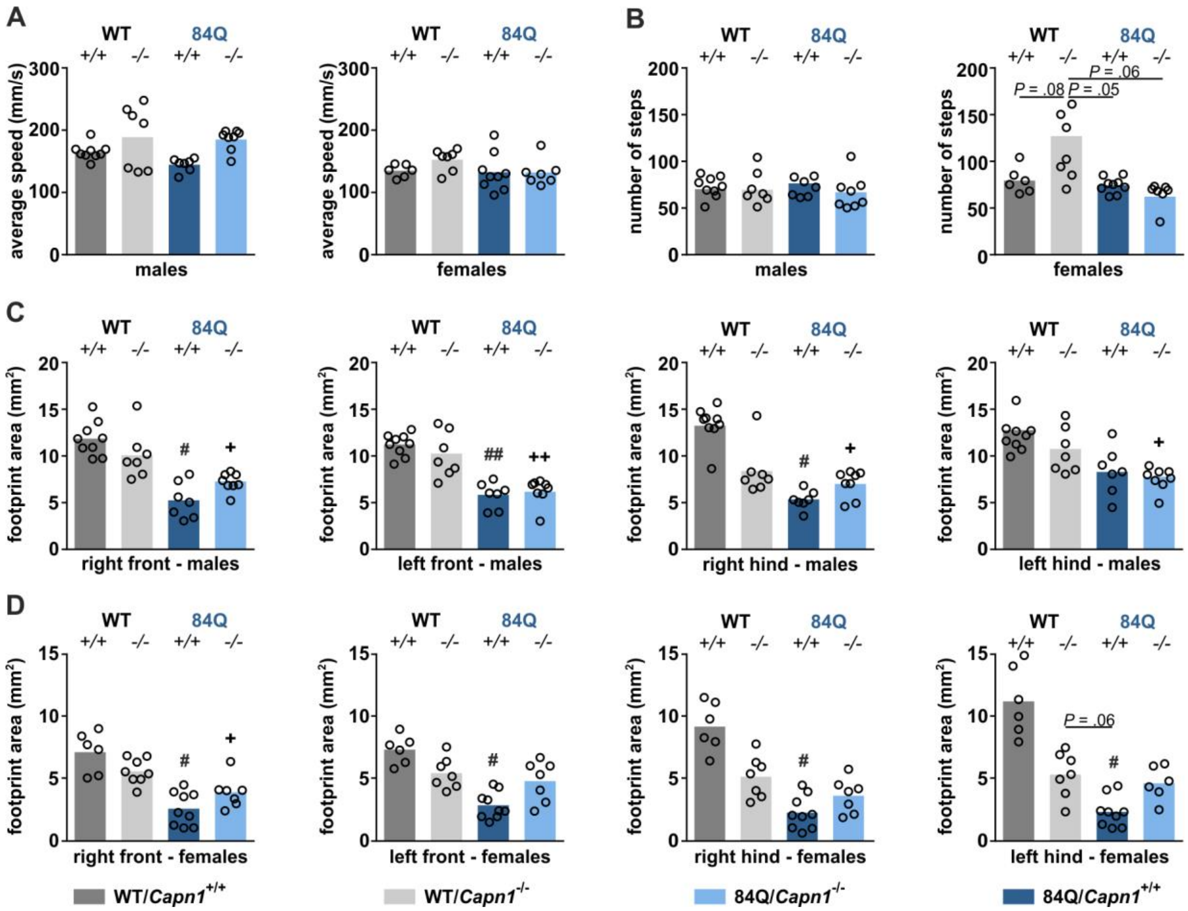


Supplementary Figure S2. Calpain-1 knockout affects the calpain system in muscle tissue

(A) Western blot of calpain-1, CAST and substrate protein α-spectrin in femoral muscle tissue of 3 months old mice. Citrate synthase (CS) served as a loading control.

(B) Western blot of ataxin-3 expression and cleavage in femoral muscle tissue of 3 months old mice. Ataxin-3 fragmentation was detected using the centre-binding antibody 1H9. Ataxin-3 breakdown products (bdp) are indicated with black and blue arrowheads (labelled with 1a, 1b, 2a, and 2b). Blue arrowheads indicate ataxin-3 fragments specifically formed in YAC84Q animals. Citrate synthase served as a loading control.

WT/*Capn1*$^{+/+}$: n = 2; WT/*Capn1*$^{-/-}$: n = 2; 84Q/*Capn1*$^{+/+}$: n = 2; 84Q/*Capn1*$^{-/-}$: n = 2. White arrowheads indicate full-length proteins, black arrowheads protein fragments. White bullets indicate heavy (◦◦) and light (◦) IgG chains. eg = endogenous ataxin-3, ex = polyQ-expanded ataxin-3.



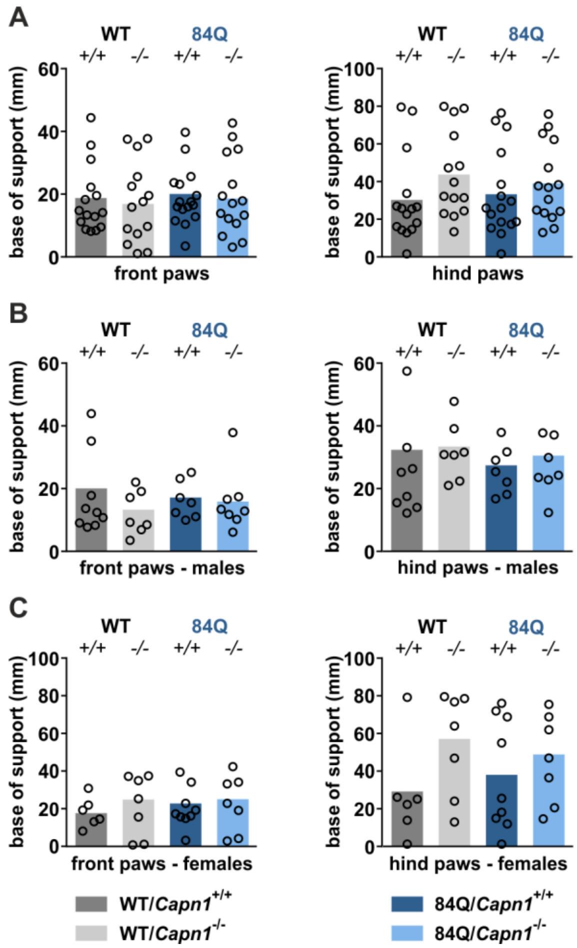
Supplementary Figure S3. Gait analyses show reduced footprint pattern in male and female YAC84Q mice at 15 months of age

(A) Measurement of mouse running speed by sex using CatWalk gait analysis at 15 months of age.

(B) Step number assessment by sex using CatWalk gait analysis at 15 months of age.

(C, D) Footprint area determination of all four paws by sex using CatWalk gait analysis at 15 months of age.

Columns represent means (WT/*Capn1*$^{+/+}$: n = 9 ♂ / 6 ♀; WT/*Capn1*$^{-/-}$: n = 7 ♂ / 7 ♀; 84Q/*Capn1*$^{+/+}$: n = 7 ♂ / 9 ♀; 84Q/*Capn1*$^{-/-}$: n = 8 ♂ / 7 ♀). $^{\#}$: WT/*Capn1*$^{+/+}$ vs 84Q/*Capn1*$^{+/+}$; $^{+}$: WT/*Capn1*$^{+/+}$ vs 84Q/*Capn1*$^{-/-}$; $^{\#/+}P \leq 0.05$, $^{\#\#/++}P \leq 0.01$, two-way ANOVA with Bonferroni post-test.

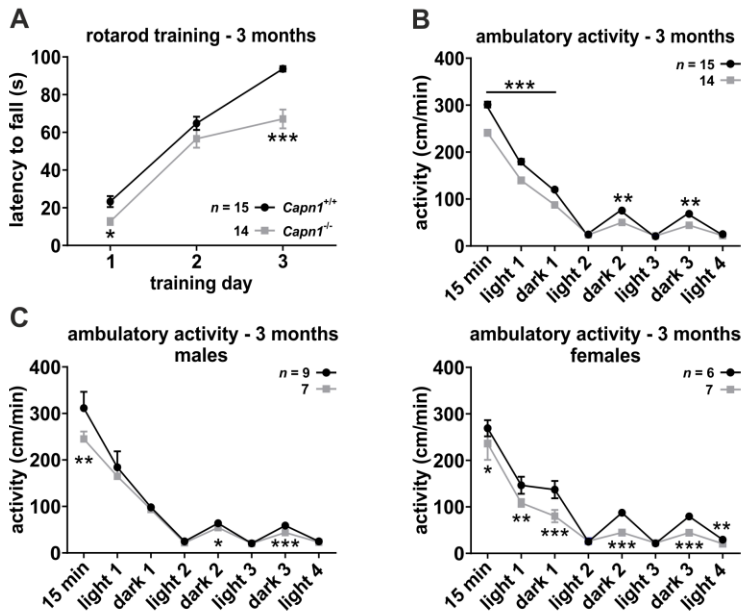


Supplementary Figure S4. No differences in base of support for front and hind paws at 15 months of age

(A) Base of support analysis for all mice using CatWalk gait analysis at 15 months of age (WT/*Capn1*$^{+/+}$: n = 15; WT/*Capn1*$^{-/-}$: n = 14; 84Q/*Capn1*$^{+/+}$: n = 16; 84Q/*Capn1*$^{-/-}$: n = 15).

(B, C) Base of support analysis by sex using CatWalk gait analysis at 15 months of age (WT/*Capn1*$^{+/+}$: n = 9♂ / 6♀; WT/*Capn1*$^{-/-}$: n = 7♂ / 7♀; 84Q/*Capn1*$^{+/+}$: n = 7♂ / 9♀; 84Q/*Capn1*$^{-/-}$: n = 8♂ / 7♀. $^{\#}$: WT/*Capn1*$^{+/+}$ vs 84Q/*Capn1*$^{+/+}$).

Columns represent means.



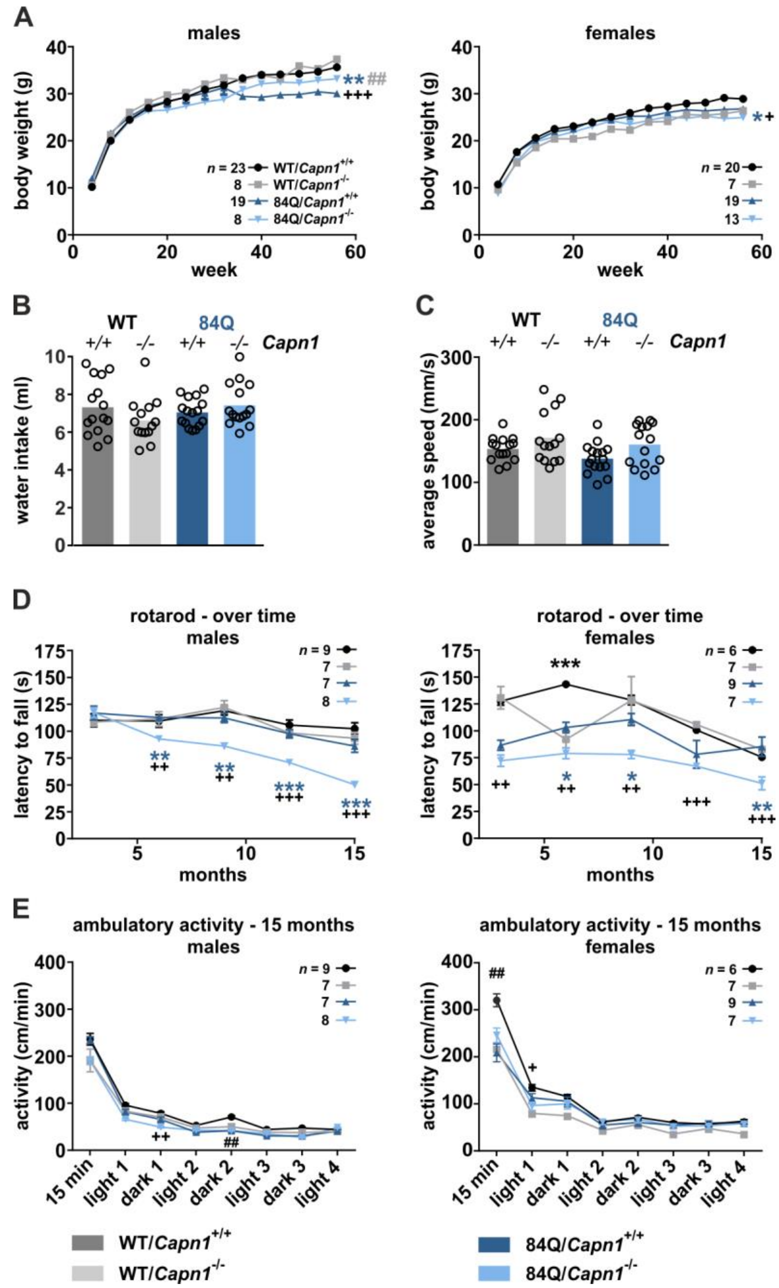
Supplementary Figure S5. Impaired learning in rotarod performance test and reduced ambulatory activity in *Capn1*$^{-/-}$ mice at 3 months of age

(A) Analysis of learning behaviour in rotarod performance test in the first training sessions at 3 months of age.

(B) Ambulatory activity assessment during light and dark phases using the home cage-based LabMaster system at 3 months of age.

(C) Ambulatory activity assessment divided by sex during light and dark phases using the home cage-based LabMaster system at 3 months of age.

Data points represent means ± SEM. Number of animals is indicated in each panel. *: WT/*Capn1*$^{+/+}$ vs WT/*Capn1*$^{-/-}$; *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$; two-way ANOVA with Bonferroni post-test.



Supplementary Figure S6. Further sex-dependent and –independent characteristics of mice until an age of 15 months of age.

(A) Sex-dependent body weight analysis with measurements performed every second week until the age of 56 weeks. Number of animals is indicated in each panel. $*^{(blue)}$: 84Q/*Capn1*$^{+/+}$ vs 84Q/*Capn1*$^{-/-}$, $^{\#(black)}$: WT/*Capn1*$^{+/+}$ vs 84Q/*Capn1*$^{+/+}$, $^{\#(grey)}$: WT/*Capn1*$^{-/-}$ vs 84Q/*Capn1*$^{-/-}$, $^{+}$: WT/*Capn1*$^{+/+}$ vs 84Q/*Capn1*$^{-/-}$; $*^{/+}P \leq 0.05$, $**^{/\#\#}P \leq 0.01$, $^{+++}P \leq 0.001$; ANOVA with Bonferroni post-test.

(B) Home cage-based LabMaster analysis of water intake at 15 months of age (WT/*Capn1*$^{+/+}$: n = 15; WT/*Capn1*$^{-/-}$: n = 14; 84Q/*Capn1*$^{+/+}$: n = 16; 84Q/*Capn1*$^{-/-}$: n = 15).

(C) Measurement of mouse running speed using CatWalk gait analysis at 15 months of age (WT/*Capn1*$^{+/+}$: n = 15; WT/*Capn1*$^{-/-}$: n = 14; 84Q/*Capn1*$^{+/+}$: n = 16; 84Q/*Capn1*$^{-/-}$: n = 15).

(D) Analysis of motor deficits by sex using the rotarod performance test as assessed every 3 months between 3 and 15 months of age. Number of animals is indicated in each panel.

(E) Ambulatory activity assessment by sex during light and dark phases using the home cage-based LabMaster system at 15 months of age. Number of animals is indicated in each panel.

Data points show means ± SEM and columns represent means. $*^{(black)}$: WT/*Capn1*$^{+/+}$ vs WT/*Capn1*$^{-/-}$, $*^{(blue)}$: 84Q/*Capn1*$^{+/+}$ vs 84Q/*Capn1*$^{-/-}$, $^{\#}$: WT/*Capn1*$^{+/+}$ vs 84Q/*Capn1*$^{+/+}$, $^{+}$: WT/*Capn1*$^{+/+}$ vs 84Q/*Capn1*$^{-/-}$; $*^{/+}P < 0.05$, $**^{/\#\#/++}P < 0.01$, $***^{/+++}P < 0.001$; two-way ANOVA with Bonferroni post-test.